

67

25

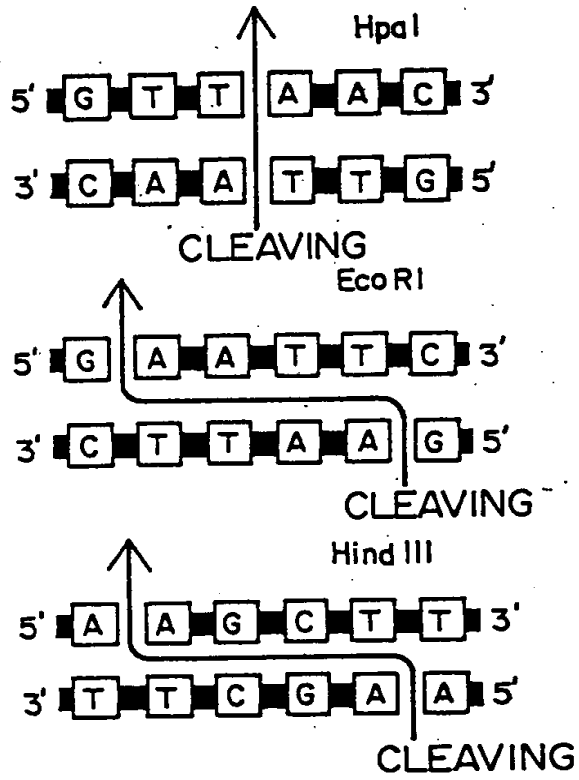


FIG. 1

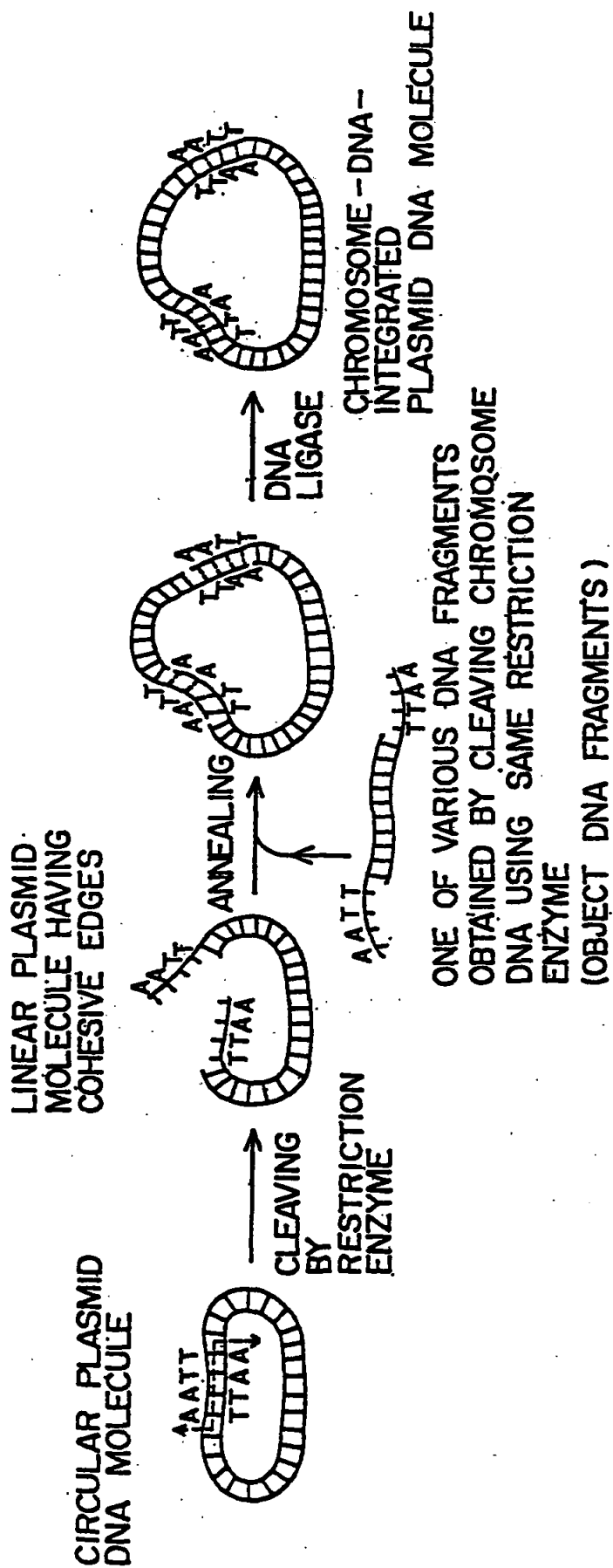


FIG. 2

09785269-052401
104250-69258760

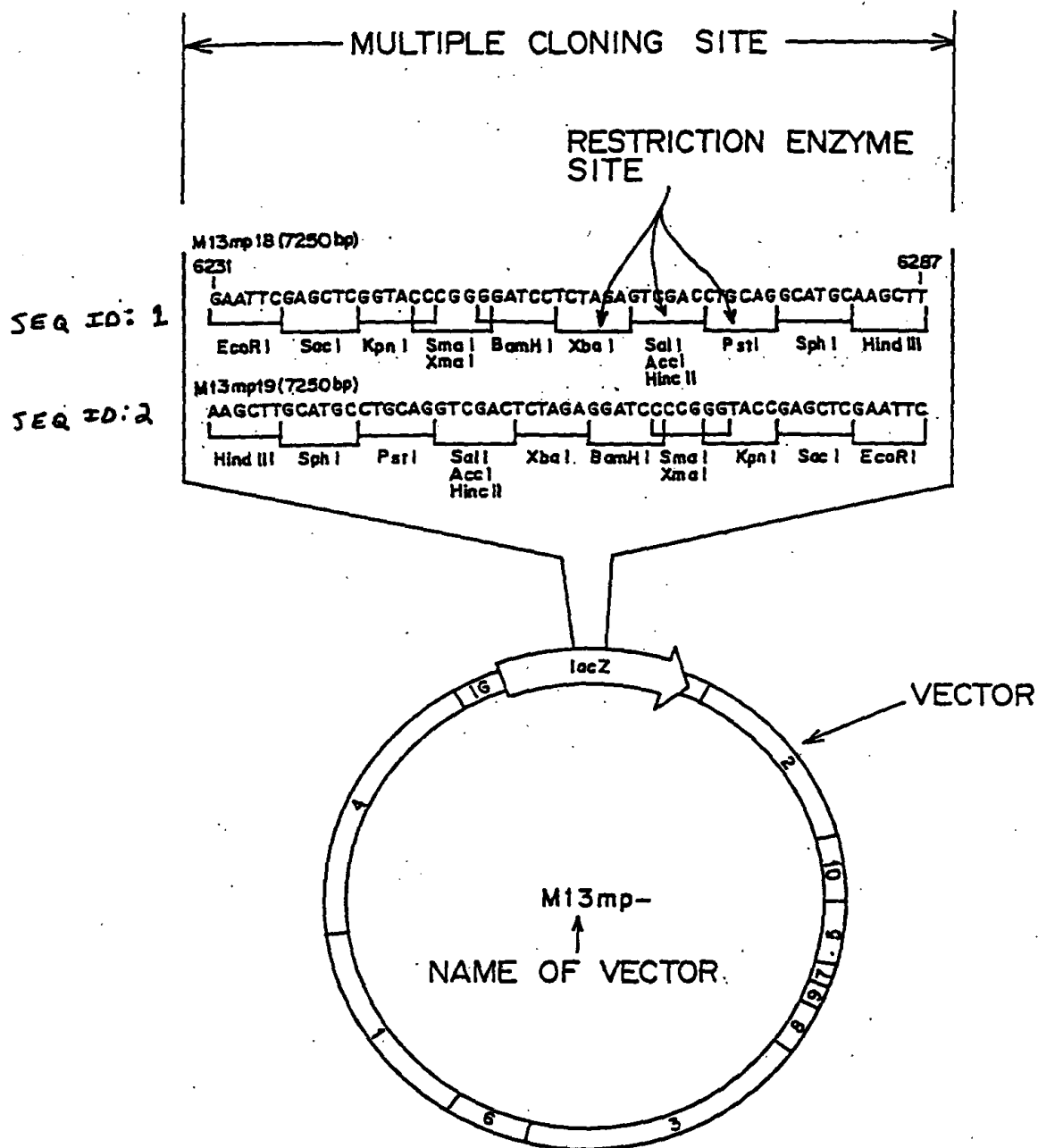
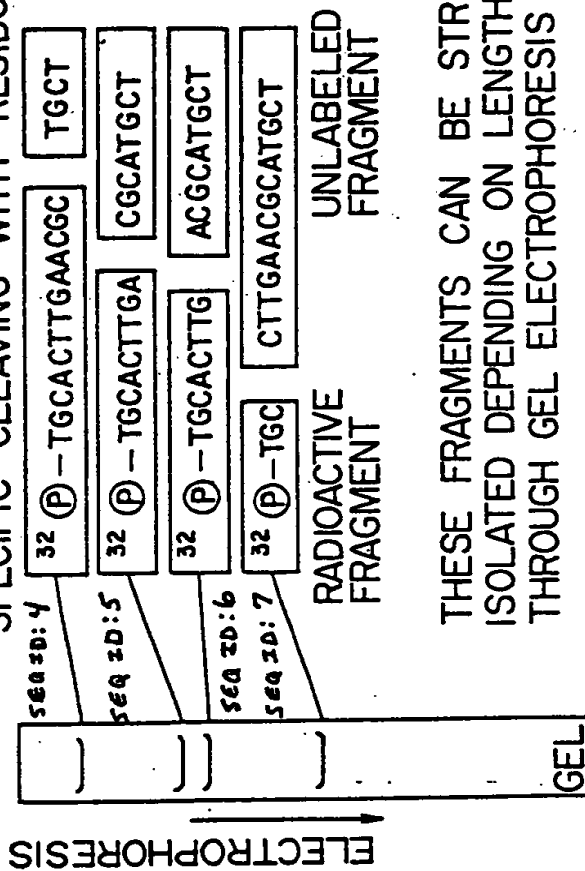


FIG. 3

DNA FRAGMENT LABELED WITH ^{32}P AT 5' EDGE

SEQ ID: 3 ^{32}P - TGCACCTTGAACGCATGCT

RADIOACTIVE FRAGMENTS OF VARIOUS LENGTHS THROUGH CHEMICAL PROCESS OF SPECIFIC CLEAVING WITH RESIDUAL BASE A



THESE FRAGMENTS CAN BE STRICTLY ISOLATED DEPENDING ON LENGTH THROUGH GEL ELECTROPHORESIS

FIG. 4

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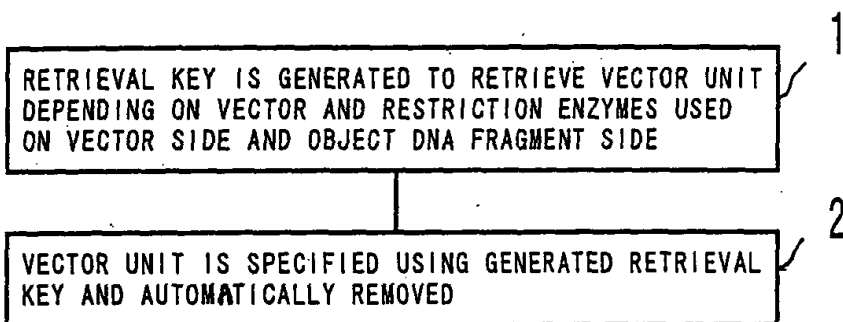
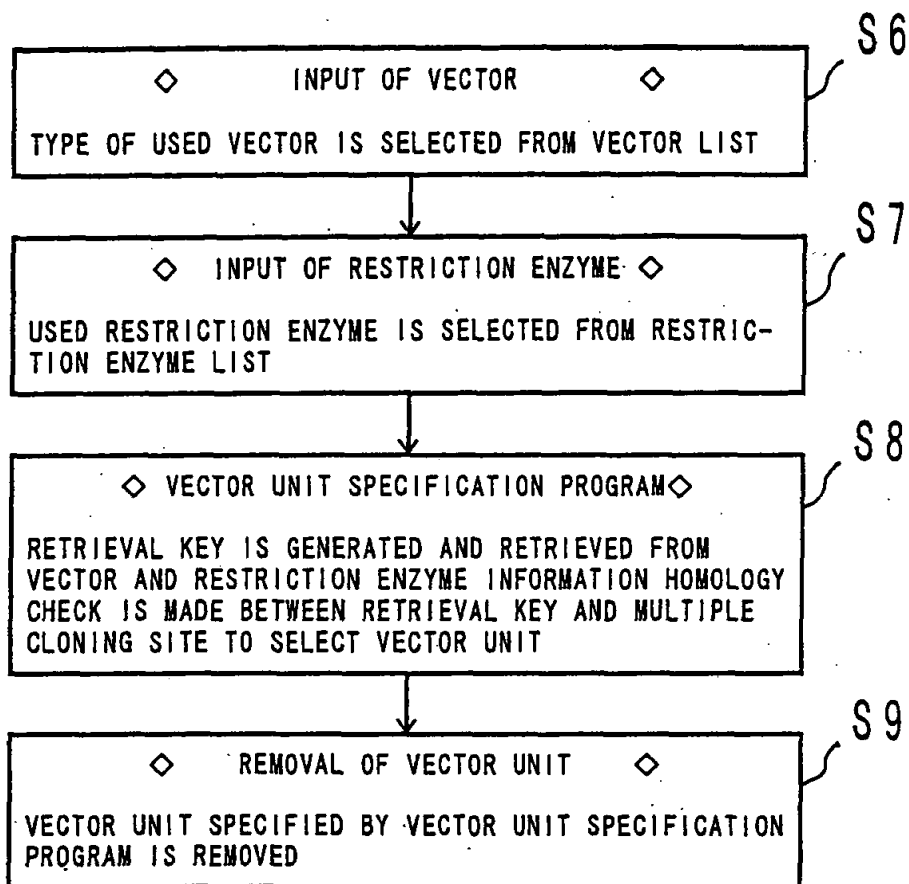


FIG. 5

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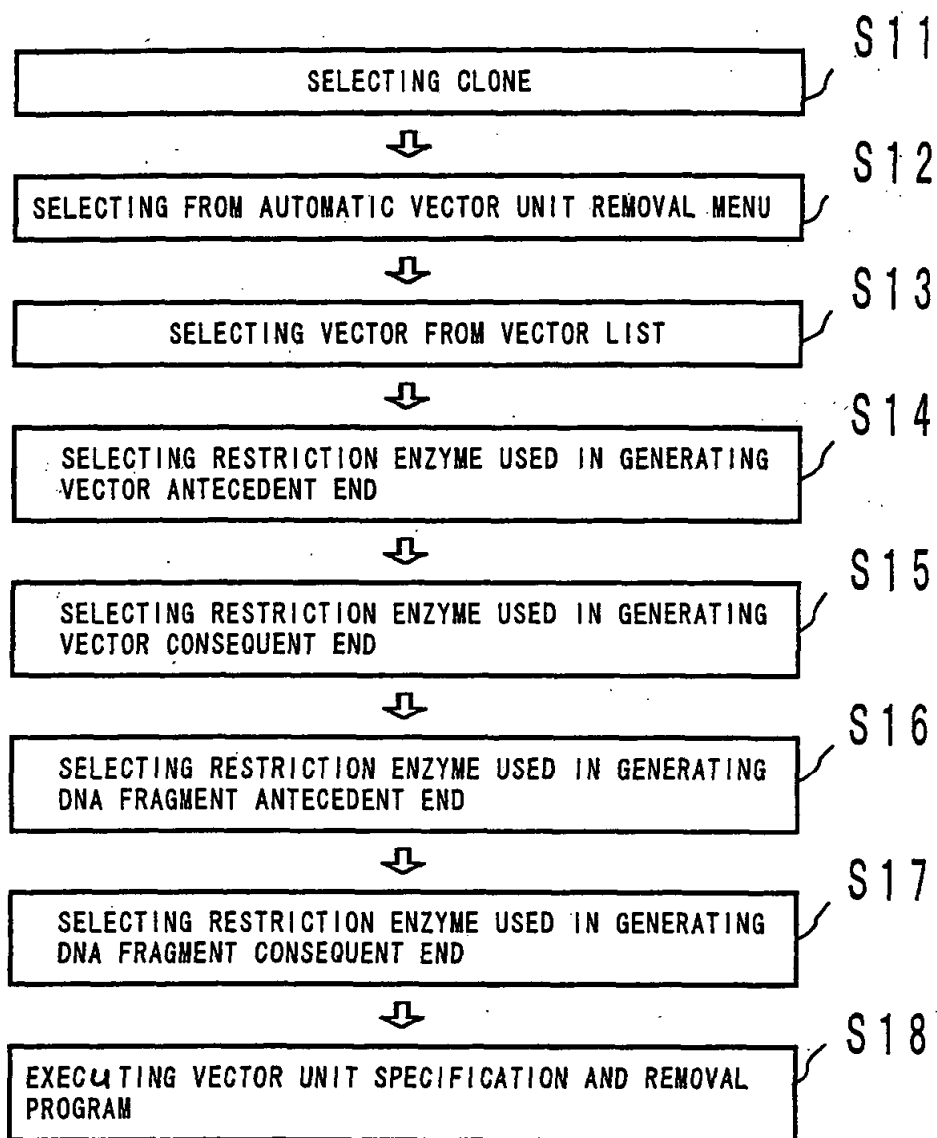


FIG. 7

09785269-052401

M1 3MP 1 8
M1 3MP 1 9
PBR 3 2 2
PSL 1 1 8 0
PSL 1 1 9 0
PT 7 T 3 1 8 U
PT 7 T 3 1 9 U
PT Z 1 8 R
PT Z 1 9 R
PUC 1 8
PUC 1 9, ETC.

FIG. 8

VECTOR DB FORMAT

```

>ID
PUC18
>SEQ 10: 8
TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGAT
GCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGCGGGCTGGCTTAACATGCGGCATCAGA
GCAGATTGTACTGAGAGTGACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGCC
ATTGCGCATTGAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGG
GGATGTGCTGCAAGGCGATTAAAGTTGGGAACGCCAGGGTTTTCCAGTCACGACGTTGTAACGACGCGCCAGTGCCAA
GCTTGCAATGCGCTGAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCT
GTGTGAAATTGTTATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATG
AGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCGCTTTCCAGTCGGGAAACCTGTGTCGCCAGCTGCATTAAT
GAATCGGCCAACGCGCGGGGAGAGGCGGTTTTGCGTATTGGGCGCTCTTCGCTTCTCGCTCACTGACTGCTGCGCTCG
GTGTTGCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGG
AAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGCTGGCGTTTTTCCATAGGCTCC
GCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCG
TTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCTGCCGCTTACCGEATACCTGTCCGCTTTCTCCCTTC
GGGAAGCGTGGCGCTTTCTCAAAGCTCAGCTGTAGGTATCTCAGTTCCGTTGTAGGTGCTTCGCTCCAAGCTGGGCTGTG
TGCACGAACCCCCCGTTGAGCCGACCGCTGCGCCTTATCCGTTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGAC
TTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTG
GTGGCCTAACTACGGCTACACTAGAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAGAGAG
TTGGTAGCTCTTGATCCGGCAAACAAACACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGA
AAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGAT
TTTGGTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGTA
TATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTGTTCA
TCCATAGTTGCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGAT
ACCGCGAGACCCACGCTCACC GGCTCCAGATTTATCAGCAATAAACCCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTC
CTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTG
CGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTGCTCCGTTCCCA
ACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCCCTCGATCGTTGTGAGAA
GTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTGATGCCATCCGTAAGATGC
TTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTC
AATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATATTGGAACGTTCTTCGGGGCGAAAACTCT
CAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCCACTGATCTTCAGCATCTTTTACTTTC
ACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAATGTTGAAT
ACTCATACTCTTCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTA
TTAGAAAAATAAATAAATAGGGGTTCCGCGCACATTTCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATC
ATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTC
>MULTI
399..450

```

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FIG. 9

(* INDICATES MULTIPLE CLONING SITE)

SEQ ID: 9 GTGCCAAGCTTGATGCCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAAT

SEQ ID: 10 AAGCTT⇒HIND III

SEQ ID: 11 GCATGC⇒SPH I

SEQ ID: 12 CTGCAG⇒PST I

SEQ ID: 13 GTCGAC⇒SAL I, ACC I, HINC II

SEQ ID: 14 TCTAGA⇒XBA I

SEQ ID: 15 GGATCC⇒BAMH I

SEQ ID: 16 CCCGGG⇒SMA I, XMA I

SEQ ID: 17 GGTACC⇒KPN I

SEQ ID: 18 GAGCTC⇒SAC I

SEQ ID: 19 GAATTC⇒ECOR I

FIG. 10

09785269-052401

VECTOR SIDE

HIND III

SPH I

PST I

SAL I

ACC I

HINC II

XBA I

BAMH I

SMA I

XMA I

KPN I

SAC I

ECOR I

OBJECT DNA
FRAGMENT SIDE

HINDIII

SPH I

PST I

SAL I

ACC I

HINC II

XBA I

BAMH I

SMA I

XMA I

KPN I

SAC I

ECOR I

OTHER RESTRICTION
ENZYME

. . .

FIG. 11

09785269 052401
T04250 69258260

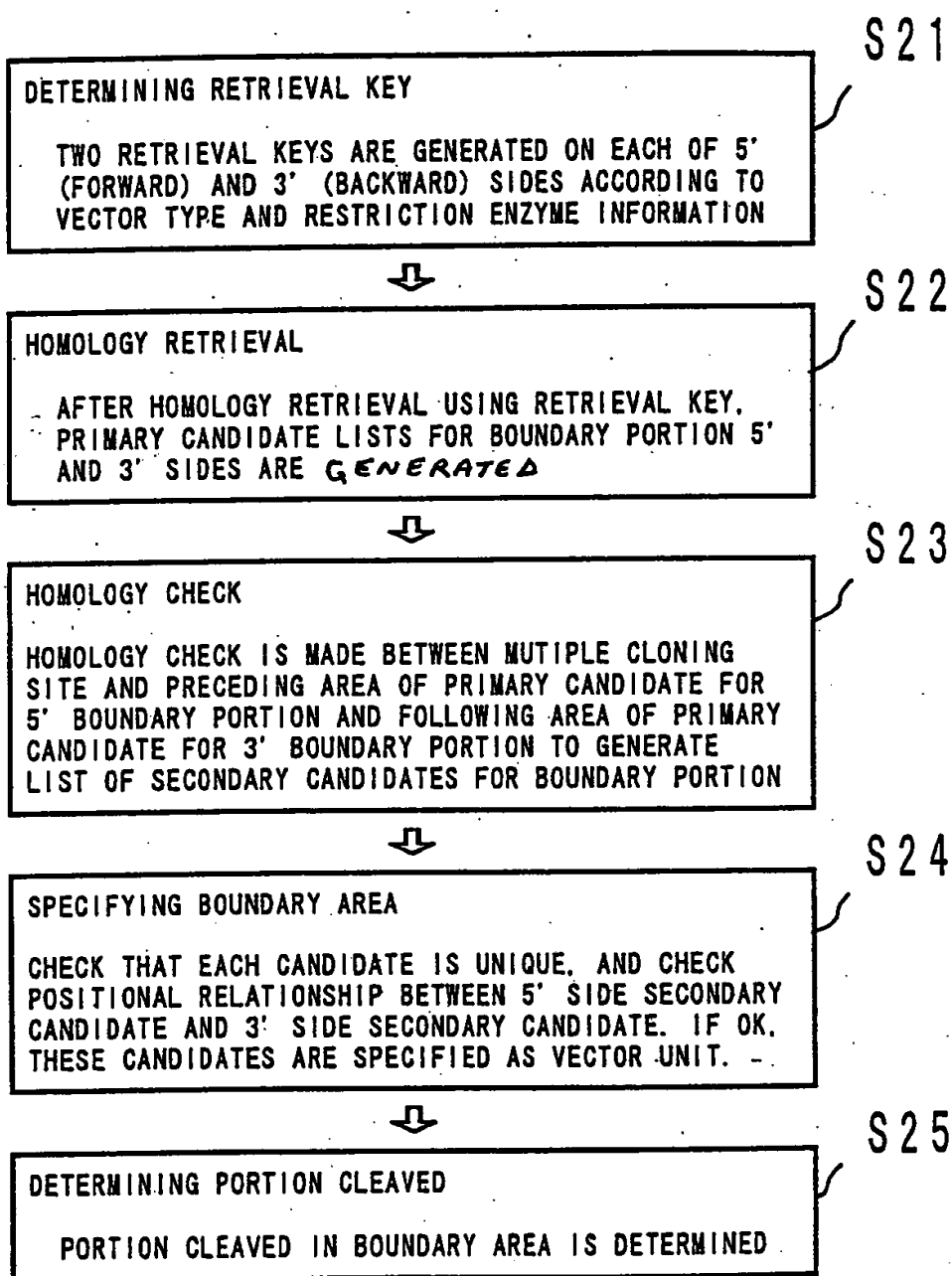


FIG. 12

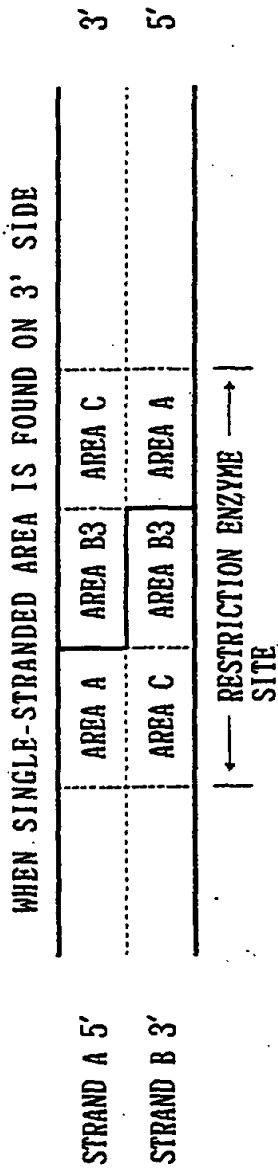


FIG. 13A

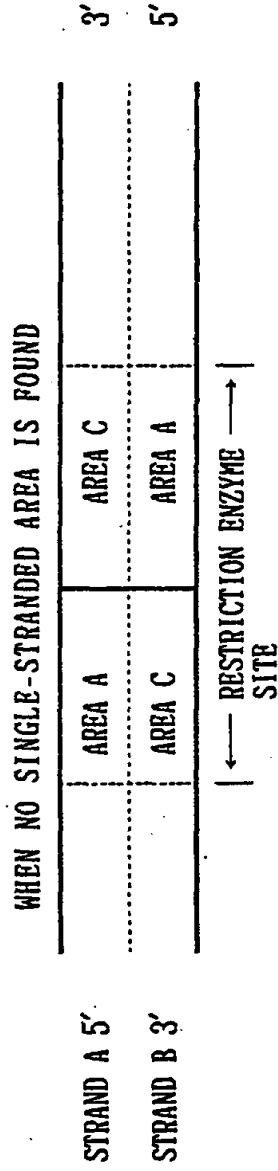


FIG. 13B

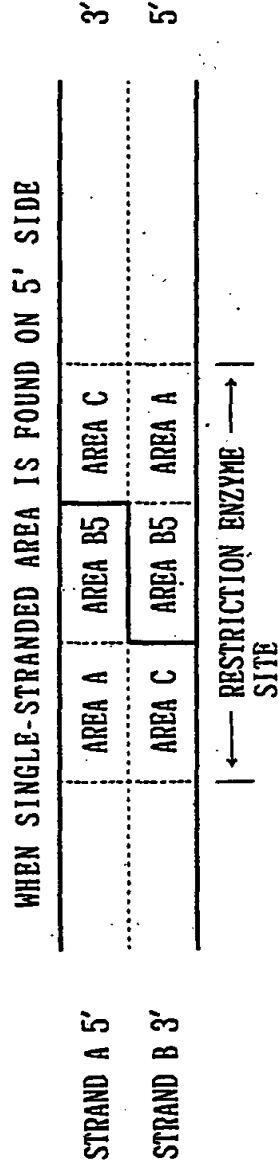


FIG. 13C

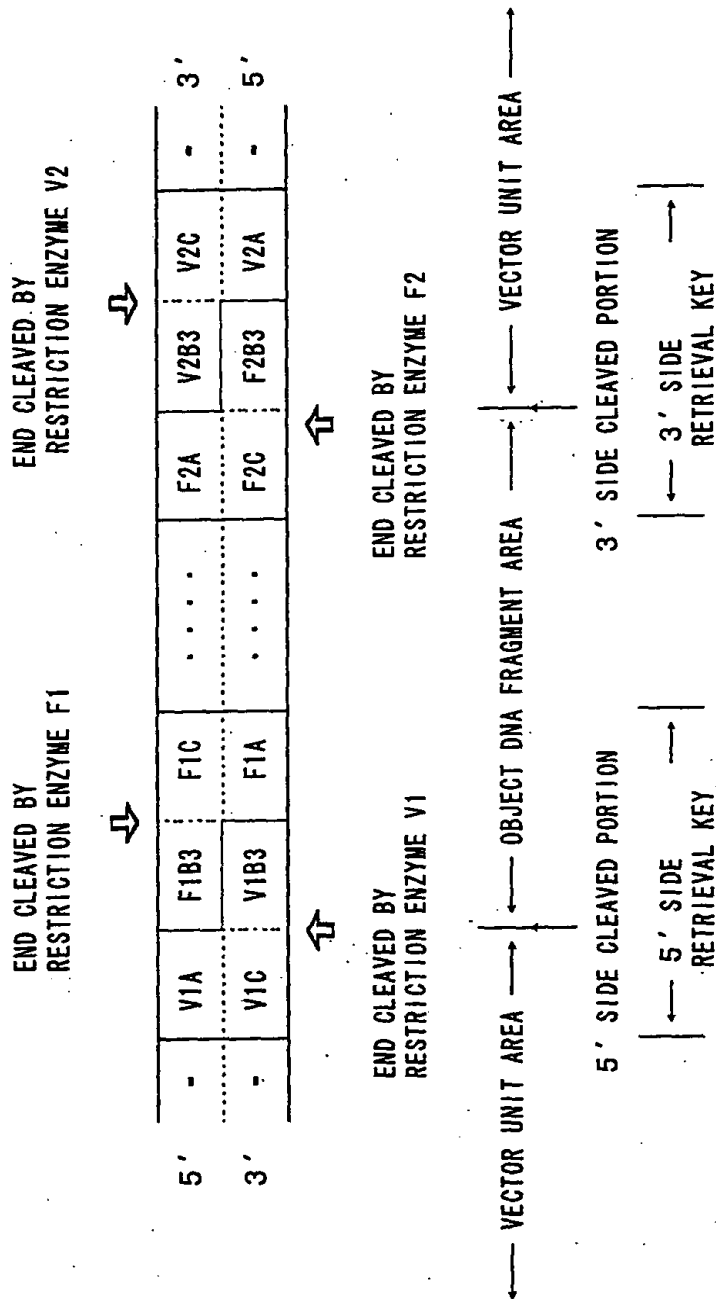


FIG. 14

09785269-052401

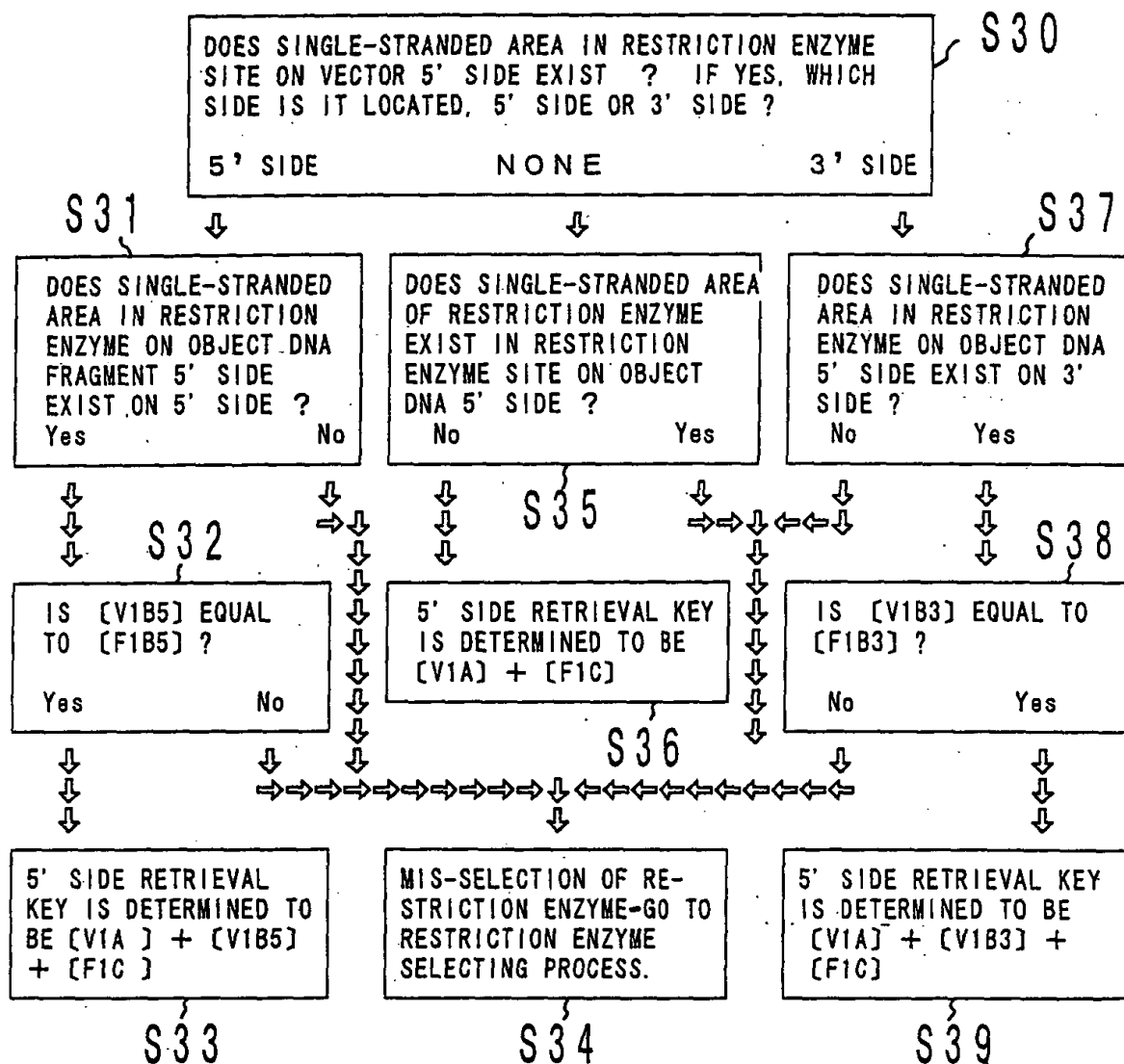


FIG. 15

09785269-052401

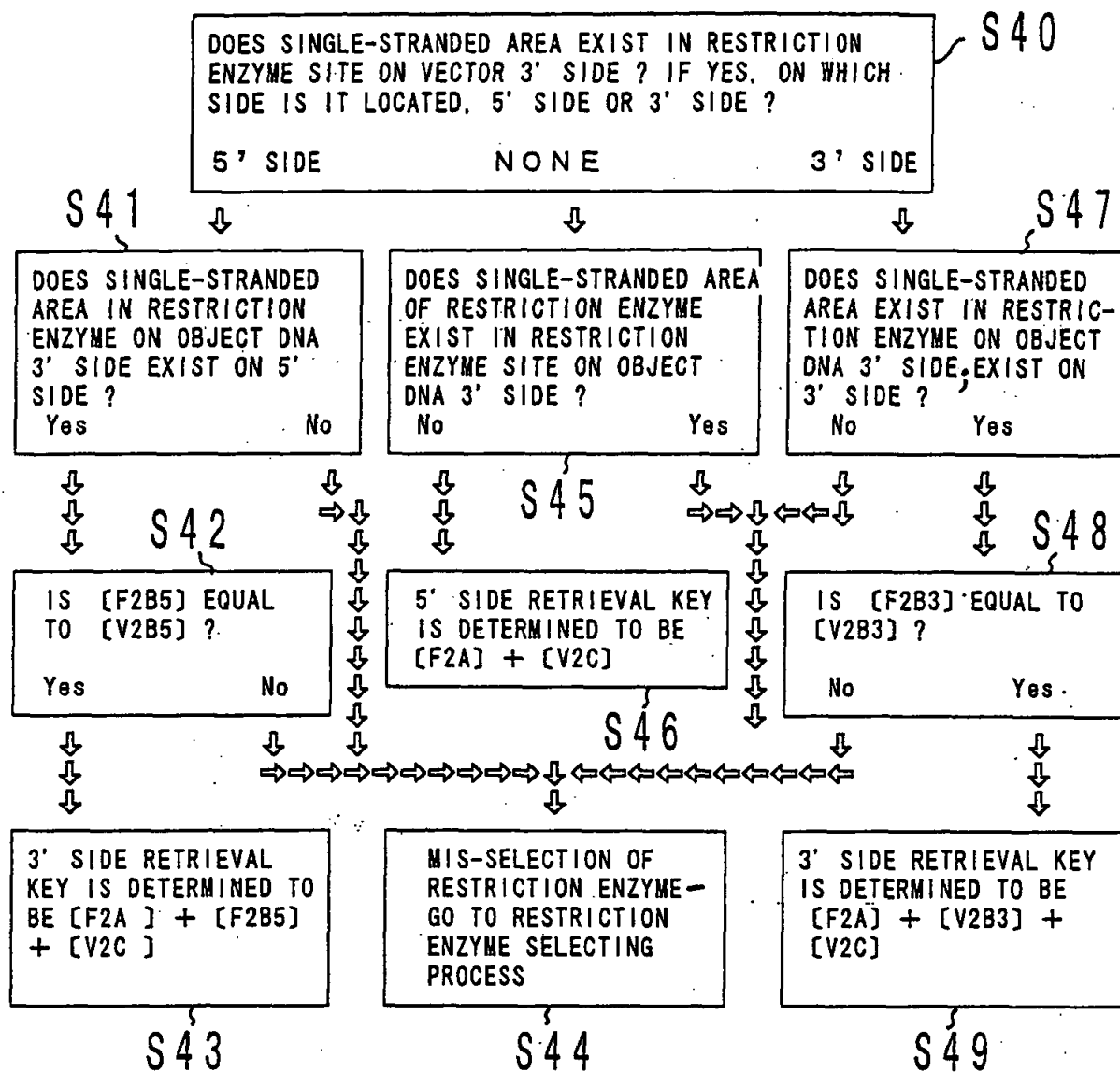


FIG. 16

WHEN HIND III IS SPECIFIED ON VECTOR 5' SIDE
 XBA I IS SPECIFIED ON VECTOR 3' SIDE, HIND III IS
 SPECIFIED ON OBJECT DNA 5' SIDE, AND XBA I IS
 SPECIFIED ON OBJECT DNA 3' SIDE

(**** INDICATES RESIDUAL MULTIPLECLONING SITE
 (++++ INDICATES AN OBJECT DNA FRAGMENT

```

****                                ****
GTGCAAGCTT+++++TCTAGAGGATCCCCGGTACCGAGCTCGAATTCGTAAT
  AAGCTT                                TCTAGA
    ↑                                ↑
  
```

5' SIDE RETRIEVAL KEY
 (IN THIS EXAMPLE,
 HIND III SITE)

9' SIDE RETRIEVAL KEY
 (IN THIS EXAMPLE, XBA I SITE)

FIG. 17

09785269-052401

09785269-052401

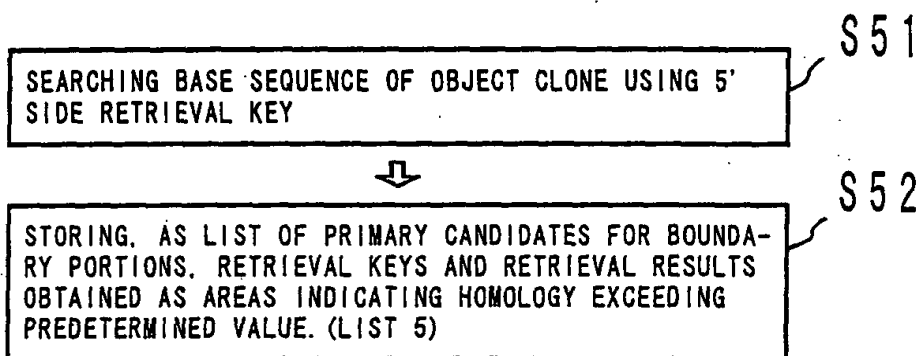


FIG. 18

09785269-052404
107250-69258760

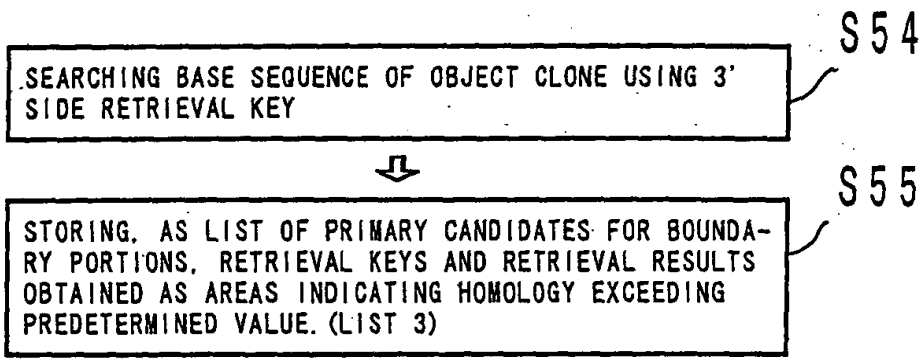


FIG. 19

09785269-052401

DEFINING, IN MULTIPLE CLONING SITE OF VECTOR, RESTRICTION ENZYME SITE USED IN SHEARING 5' SIDE IN MULTIPLE CLONING SITE OF VECTOR AND AREA OUTSIDE ON 5' SIDE AS 5' SIDE RESIDUAL MULTIPLE CLONING SITE (5MCS)

S 6 1



WHEN VECTOR DB CONTAINS BASE SEQUENCE OTHER THAN MULTIPLE CLONING SITE, SUM OF 5MCS AND 5 BASES ON 5' SIDE FROM 5MCS IS DEFINED AS 5' SIDE RESIDUAL VECTOR AREA (5VA). IF VECTOR DB CONTAINS ONLY BASE SEQUENCE OF MULTIPLE CLONING SITE IN VECTOR DB, THEN 5 MCS IS 5VA.

S 6 2

{ A HOMOLGY CHECK IS MADE ACCORDING TO FOLLOWING FLOWCHART ON ALL ELEMENTS IN PRIMARY CANDIDATES FOR BOUNDARY PORTIONS (LIST 5) OBTAINED IN 5' SIDE HOMOLGY RETRIEVAL }

DEFINING EACH CANDIDATE IN LIST 5 AND SEQUENCE AREA OUTSIDE ON 5' SIDE AS HOMOLGY CHECK AREA (5HCA) FOR CORRESPONDING CANDIDATE

S 6 3



COMPARING NUMBER OF BASES IN 5' SIDE RESIDUAL VECTOR AREA (5VA), NUMBER OF BASES OF 5HCA, AND NUMBER OF BASES 20, AND DEFINING SMALLEST NUMBER OF BASES AS NUMBER OF BASES FOR USE IN HOMOLGY CHECK (HCB)

S 6 4



EXTRACTING HCB BASES FROM 3' SIDE OF 5VA TO CHECK HOMOLGY TO HCB BASES ON 3' SIDE OF 5HCA

S 6 5



WHEN CONSTANT HOMOLGY IS OBTAINED, EXTRACTED BASES ARE DEFINED AS SECONDARY CANDIDATES FOR 5' SIDE BOUNDARY PORTIONS.

S 6 6

F I G . 2 0

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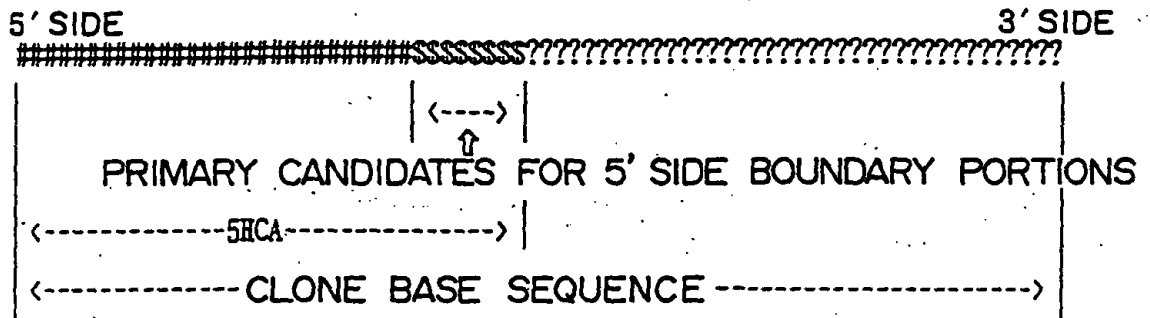


FIG. 21

09785269-052401

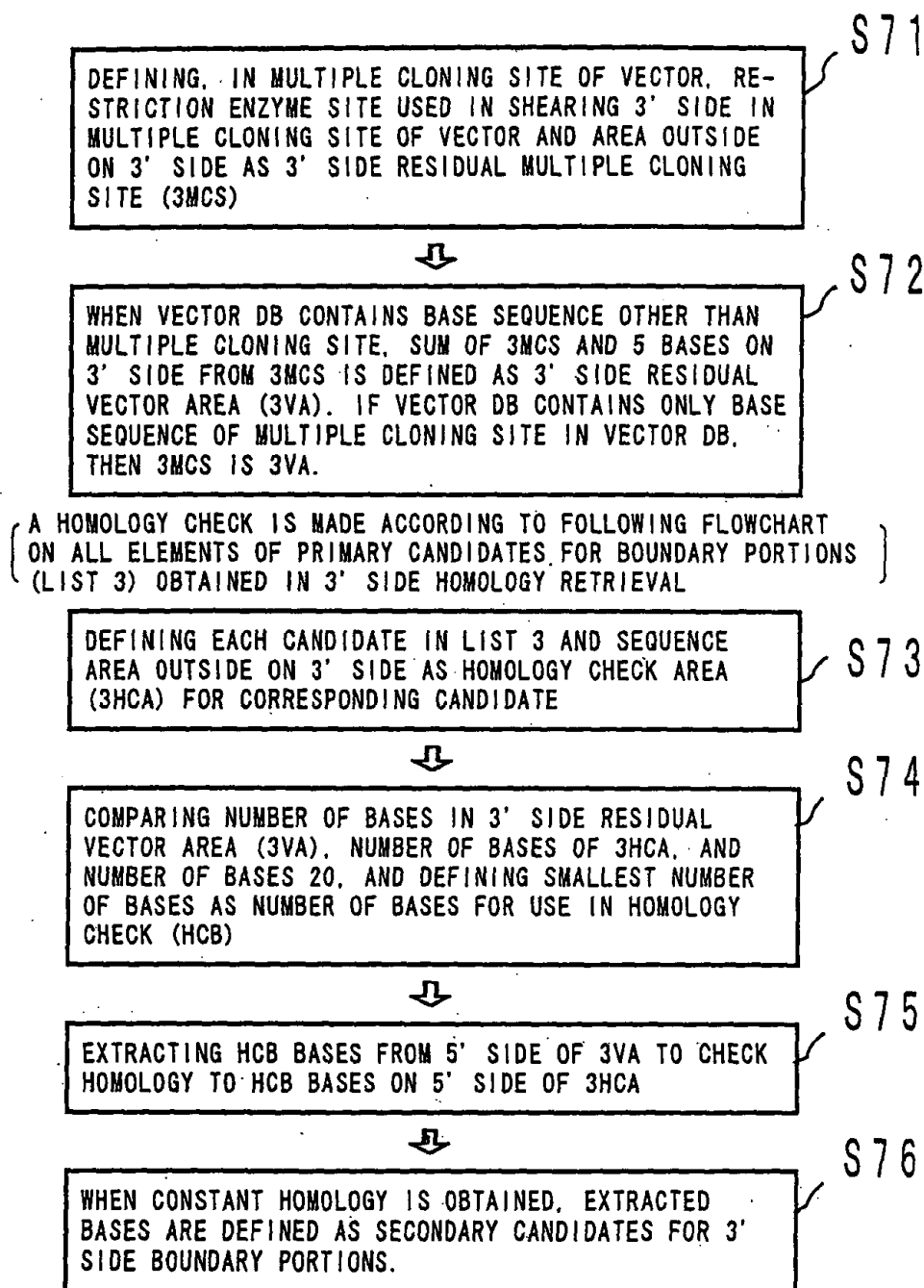


FIG. 22

09785269.052401
T04250" 69258460

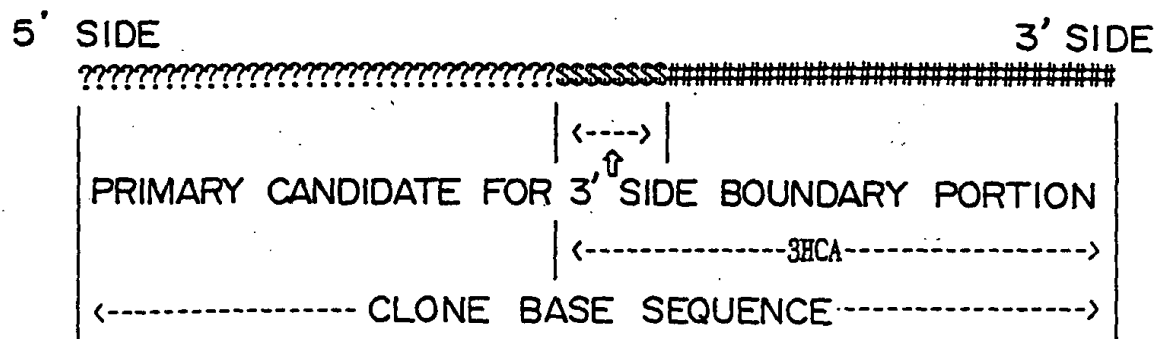


FIG. 23

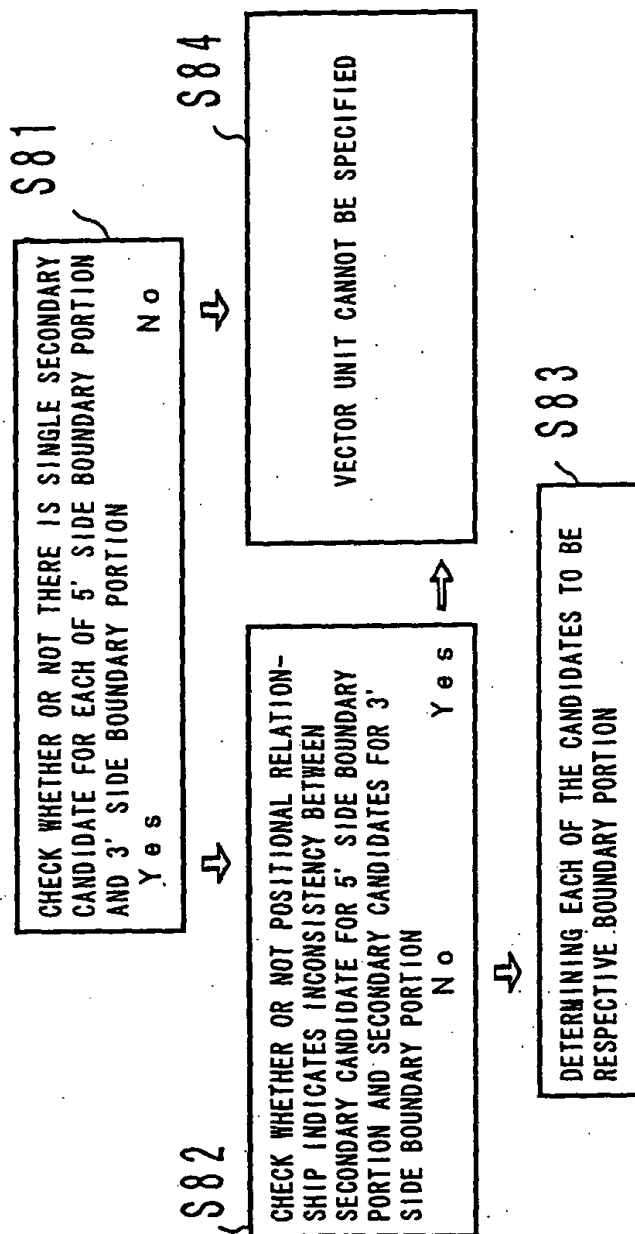


FIG. 24

FIG. 25

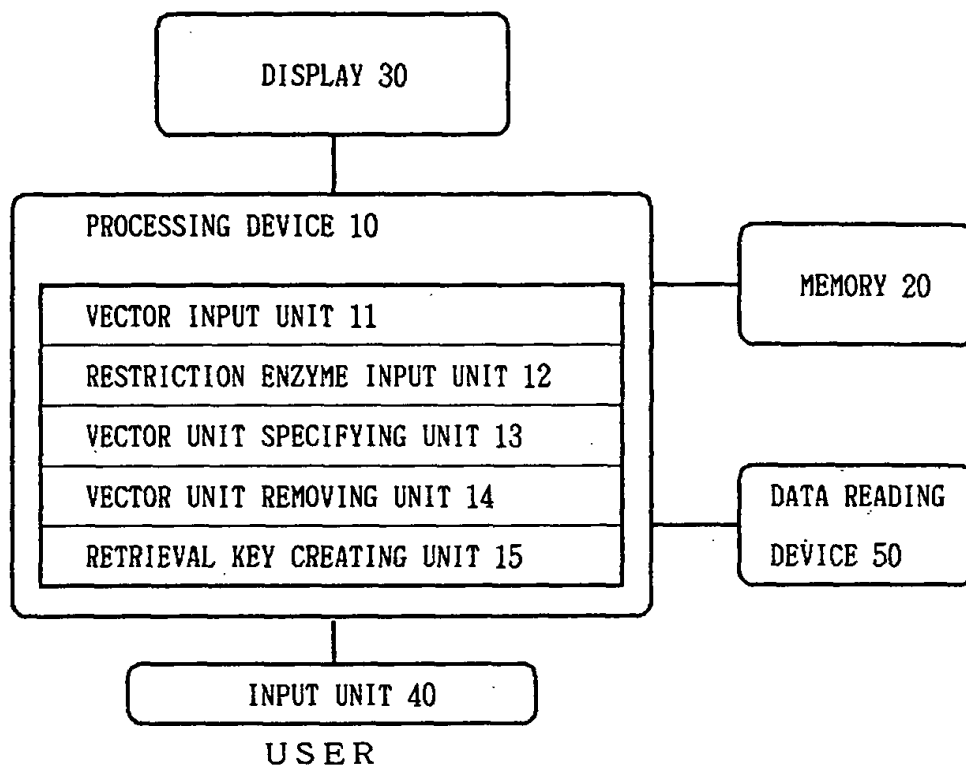


FIG. 25